IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
Group Art Unit - 1636

In re

Electronically filed by Tracy Bruesewitz on March 7, 2011.

Patent Application of

Michael R. Slater

Application No. 10/702,228

Confirmation No.: 8004

Filed: November 5, 2003

Examiner: Nancy Treptow Vogel

"VECTORS FOR DIRECTIONAL CLONING"

DECLARATION OF MICHAEL R. SLATER UNDER 37 CFR § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Michael R. Slater, do declare and state the following:

1. I have personal knowledge of the following facts and I make this declaration in support of the prosecution of U.S. Patent Application Serial No. 10/702,228 before the United States Patent and Trademark Office.

2. I obtained a Ph.D. in 1986 from the University of Wisconsin, Madison. I have worked as Senior Research Scientist at Promega Corporation since 1992. Attached to this Declaration as Appendix B is a copy of my Curriculum Vitae.

3. I understand that in an Office action dated October 7, 2010 claims 75-78, 80-89, and 98 were rejected as being obvious over U.S. Patent No. 6,248,569 issued to Dunn et al. in view of U.S. Patent No. 5,342,782 issued to Thach, Kappelman et al. (1995) *Gene*, 160:55-98, and the New England Biolabs Catalog.

4. I discovered that the claimed vectors work unexpectedly well in facilitating cloning without the need to purify the DNA fragment of interest. Attached as an Appendix are data showing the percent efficiencies achieved using vectors according to the claimed invention

which efficiencies were achieved without purifying the DNA fragment of interest. Random ligation of the restriction fragments present in the ligation mixture would provide an expected transfer frequency of the DNA fragment of interest of approximately 50%. Instead, we observed transfer frequencies, yielding vectors carrying the DNA fragment of interest, above 80% or even 95%. The cloning efficiencies achieved are surprising, and much higher than would be expected with this type of cloning. The claimed vectors thus achieved unexpectedly high transfer frequencies, offering significant advantages both in terms of minimizing the number of colonies that must be screened to find a desired clone and also facilitating the capture of scarce or low-yield DNA fragments of interest.

5. The table in the attached Appendix A shows the percent transfer frequencies achieved using vectors within the scope of the claims. The vectors and fragment sources were digested with the indicated restriction enzymes and ligated without intervening purification of the vector or the DNA fragment of interest. Exemplary vectors and fragment sources are also shown in the Appendix.

6. A similar protocol was used for each of the cloning reactions in the Appendix. For example, cloning reaction 1 in the Appendix was conducted using the acceptor vector pF5A cut with *Sgf*I and *Pme*I, and the DNA fragment was obtained by digesting the donor vector pF1K-LacZα with *Sgf*I and *Pme*I. The resulting restriction products were ligated, used to transform competent cells, and plated in triplicate on agar plates containing X-gal. The number of blue colonies and total colonies were counted on each plate to determine the transfer frequency of the cloning reaction.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

7 Mar 2011
Dated

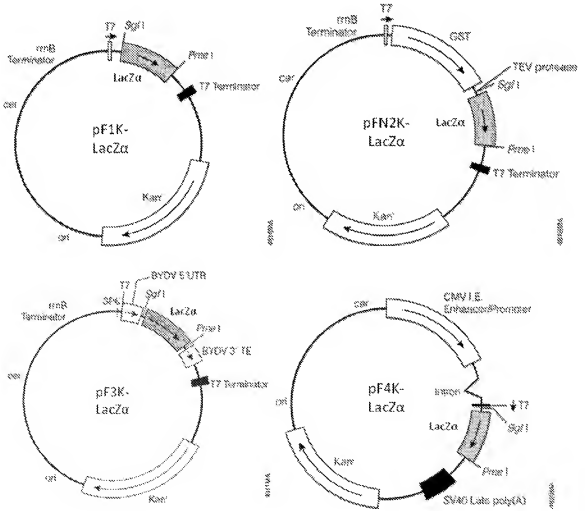
Michael R. Slater

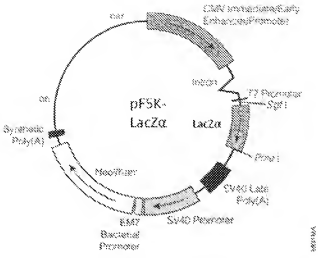
APPENDIX A OF THE DECLARATION OF MICHAEL R. SLATER UNDER 37 CFR § 1.132

Cloning Reaction	*Vector*	*First R.E. Site*	*Second R.E. Site*	*Fragment Source*	*R.E. Generating a 3' Overhang*	*Third R.E. Site*	*Total CFU*	*Transfer Frequency (% blue)*	*Mean Transfer Frequency*
1	pF5A	*Sgf*I	*Pme*I	pF1K-LacZα	*Sgf*I	*Pme*I	1078	97.3	97.6%
							600	98.3	
							646	97.1	
2	pF5A	*Sgf*I	*Pme*I	pF1K-LacZα	*Sgf*I	*Pme*I	55	90.9	76.4
							2	50.0	
							34	88.2	
3	pF5A	*Sgf*I	*Pme*I	pFN2K-LacZα	*Sgf*I	*Pme*I	118	89.8	86.6
							6	83.3	
							113	86.7	
4	pF5A	*Sgf*I	*Pme*I	pFN2K-LacZα	*Sgf*I	*Pme*I	65	95.4	90.6
							16	81.3	
							164	95.1	
5	pF5A	*Sgf*I	*Pme*I	pF3K-LacZα	*Sgf*I	*Pme*I	27	59.3	70.7
							40	70.0	
							29	82.8	
6	pF5A	*Sgf*I	*Pme*I	pF3K-LacZα	*Sgf*I	*Pme*I	99	61.6	61.2
							219	63.9	
							269	58.0	
7	pF5A	*Sgf*I	*Pme*I	pF3K-LacZα	*Sgf*I	*Pme*I	549	74.1	68.2
							136	71.3	
							44	59.1	
8	pF5A	*Sgf*I	*Pme*I	pF4K-LacZα	*Sgf*I	*Pme*I	340	84.1	88.5
							79	88.6	
							254	92.9	
9	pF5A	*Sgf*I	*Pme*I	pF4K-LacZα	*Sgf*I	*Pme*I	609	93.6	91.7
							219	87.2	
							312	94.2	
10	pF1A	*Sgf*I	*Pme*I	pF5K-LacZα	*Sgf*I	*Pme*I	36	97.2	94.3
							155	92.9	
							217	92.6	
11	pF1A	*Sgf*I	*Pme*I	pF5K-LacZα	*Sgf*I	*Pme*I	688	97.7	96.7
							294	96.9	
							487	95.5	
12	pFN2A	*Sgf*I	*Pme*I	pF5K-LacZα	*Sgf*I	*Pme*I	52	94.2	95.7
							28	96.4	
							110	96.4	
13	pFN2A	*Sgf*I	*Pme*I	pF5K-LacZα	*Sgf*I	*Pme*I	306	97.1	96.7
							194	96.9	
							159	96.2	
14	pF3A	*Sgf*I	*Pme*I	pF5K-LacZα	*Sgf*I	*Pme*I	265	71.3	75.9
							310	82.6	
							248	73.8	
15	pF3A	*Sgf*I	*Pme*I	pF5K-LacZα	*Sgf*I	*Pme*I	498	69.9	72.9
							741	75.7	
							934	73.0	

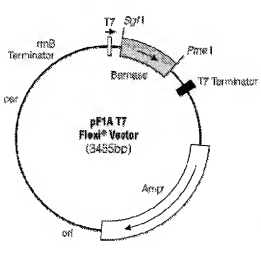
Cloning Reaction	*Vector*	*First R.E. Site*	*Second R.E. Site*	*Fragment Source*	*R.E. Generating a 3' Overhang*	*Third R.E. Site*	*Total CFU*	*Transfer Frequency (% blue)*	*Mean Transfer Frequency*
16	pF4A	*Sgf*I	*Pme*I	pF5K-LacZα	*Sgf*I	*Pme*I	164	98.2	97.9
							67	97.0	
							633	98.4	
17	pF4A	*Sgf*I	*Pme*I	pF5K-LacZα	*Sgf*I	*Pme*I	1182	98.7	99.2
							81	100.0	
							531	98.9	
18	pFN6A	*Sgf*I	*Pme*I	pF5K-LacZα	*Sgf*I	*Pme*I	773	98.6	99.0
							427	98.8	
							687	99.6	
19	pFC7A	*Sgf*I	*Eco*ICRI	pF5K-LacZα	*Sgf*I	*Pme*I	2654	80.6	80.7
							3171	80.4	
							2525	81.1	
20	pFC8A	*Sgf*I	*Eco*ICRI	pF5K-LacZα	*Sgf*I	*Pme*I	1685	98.5	97.6
							689	99.4	
							1966	95.0	

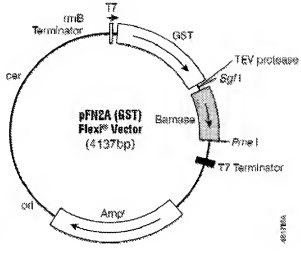
Donor Vectors with Sgf I and Pme I sites flanking the LacZ alpha peptide:

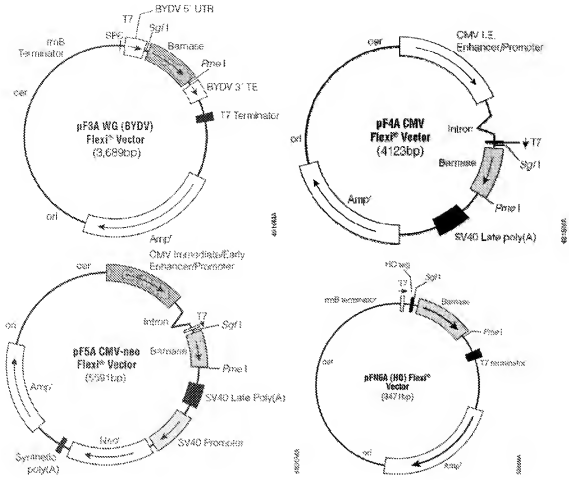




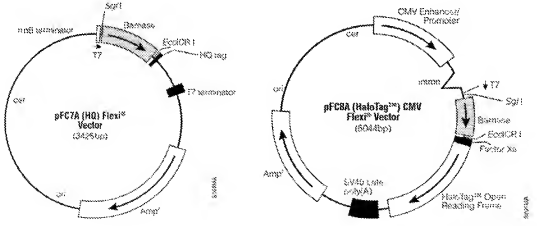
Acceptor Vectors with Sgf I and Pme I sites:







Acceptor Vectors with Sgf I and EcoICR I sites:



APPENDIX B OF THE DECLARATION OF MICHAEL R. SLATER UNDER 37 CFR § 1.132

Curriculum Vitae

Michael Ross Slater
Madison, WI

Current Position **Senior Scientist R&D, Promega Corporation, Madison, WI**

Previous Position **Senior Scientist, DNASTAR, Inc., Madison, WI**

Teaching Positions

- **Core Techniques in Protein and Genetic Engineering**

  Lecturer for two-week intensive graduate course (UW-Madison, Oncology #675) at BTCI, Madison, WI. - July 1995 to present.

- **Techniques in Bioinformatics and Comparative Genomics**

  Co-organizer/Instructor with Jeffrey Blanchard for one-week advanced workshop at the BioPharmaceutical Technology Center Institute (BTCI), Madison, WI; June 1999 to 2005, when the course became "Computational Approaches to Analyzing Microarray Data"

Education

University of Chicago	1986-1989	Postdoctoral Research Fellow
University of Wisconsin, Madison	1979-1986	**Ph.D.** Molecular Biology, 1986 Genetics minor
University of Edinburgh, Scotland	1977-1979	Diploma in Biology, 1979
California State University, Northridge	1973-1977	**B.A.** Biology 1978 Chemistry minor

Publications

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